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(54) Title: MODULATORS FOR NEW MEMBERS OF THE STEROID/THYROID SUPERFAMILY OF RECEPTORS

(57) Abstract

In accordance with the present invention, there are provided modulators for orphan member(s) of the steroid/thyroid superfamily of receptors which is related to the previously described CAR-α. Thus, compounds of the general class of androstans have been identified as modulators for a newly discovered isoform of CAR. Compounds discovered in accordance with the present invention can be employed in a variety of applications, e.g., for the modulation of processes mediated by an isoform of CAR or CAR-like species, to increase the libido of a subject (especially a subject undergoing therapy using a 5α-reductase inhibitor), in a screening assay for the presence of receptors involved in the modulation of libido, and the like. Also provided in accordance with the present invention are methods for the identification of compounds which modulate processes mediated by an isoform of CAR or CAR-like species, as well as novel compositions derived therefrom.

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Modulators for New Members of the Steroid/Thyroid Superfamily of Receptors

FIELD OF THE INVENTION

The present invention relates to intracellular receptors, and modulators therefor. In a particular aspect, the present invention relates to methods for the identification of compounds which function as modulators (or precursors thereof) for specific members of the intracellular receptor family. In other aspects, the present invention relates to various uses for the compounds so identified.

BACKGROUND OF THE INVENTION

A central problem in eukaryotic molecular biology continues to be the elucidation of molecules and mechanisms that mediate specific gene regulation. As part of the scientific attack on this problem, a great deal of work has been done in efforts to identify modulators (i.e., endogenous or exogenous inducers and/or repressors) which are capable of mediating specific gene regulation.

Although much remains to be learned about the specifics of gene regulation, it is known that ligands modulate gene transcription by acting in concert with intracellular components, including intracellular receptors and discrete DNA sequences known as hormone response elements (HREs).

As additional members of the steroid/thyroid superfamily of receptors are identified, the search for endogenous or exogenous inducers and/or repressors for such newly discovered receptors has become an important part of the effort to learn about the specifics of gene regulation.

The identification of compounds which directly or indirectly interact with intracellular receptors, and thereby affect transcription of hormone-responsive genes, would be of significant value, e.g., for therapeutic applications.

Additional novel intracellular receptors (i.e., members of the steroid/thyroid superfamily of receptors) continue to be identified. Frequently, however, the primary ligand(s) for these novel receptors can not readily be identified. Accordingly, the identification of ligands and/or modulators for such receptors is of great value.

BRIEF DESCRIPTION OF THE INVENTION

In accordance with the present invention, we have identified modulators for orphan member(s) of the steroid/thyroid superfamily of receptors which is related to the previously described constitutively active receptor-alpha (CAR-α; also known as "MB-67," see Baes et al., in Mol. and Cell. Biology 14:1544-1552 (1994)). Thus, compounds of the general class of androstans have been identified as modulators for a newly discovered isoform of CAR. Compounds discovered in accordance with the present invention can be employed in a variety of applications, e.g., for the modulation of processes mediated by an isoform of CAR or CAR-like species, to increase the libido of a subject (especially a subject undergoing therapy using a 5α-reductase inhibitor), in a screening assay for the presence of receptors involved in the modulation of libido, and the like.

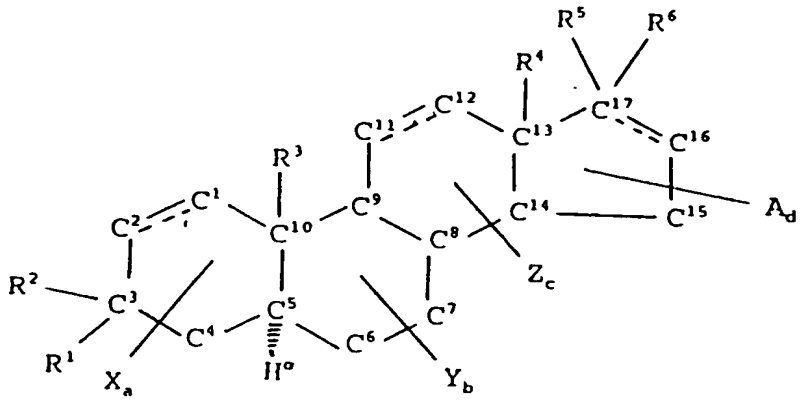
Also provided in accordance with the present invention are methods for the identification of compounds which modulate processes mediated by an isoform of CAR or CAR-like species, as well as novel compositions derived therefrom.

BRIEF DESCRIPTION OF THE FIGURE

Figure 1 illustrates the suppression of an isoform of CAR by 5α-androstane derivatives.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, there are provided methods for modulating the activity of a CAR or CAR-like isoform, said method comprising administering an effective amount of a steroid-like compound having the structure I, as set forth below:



I

wherein:

$R^1 = R^2 = O$; or R^1 = hydrogen and

R^2 is α-OR, wherein R is selected from hydrogen, lower alkyl, acyl or trimethylsilyl;

R^3 and R^4 are each independently hydrogen or lower alkyl;

$R^5 = R^6 = O$; or R^5 and R^6 are both hydrogen; or R^6 is absent when there is a double bond between C^{16} and C^{17};

X, Y, Z and A are each independently selected from hydroxy, alkoxy (of a lower alkyl group), mercapto (of a lower alkyl group), halogen, trifluoromethyl, cyano, nitro,

amino, carboxyl, carbamate, sulfonyl, or sulfonamide;

a falls in the range of 0 up to 4;

b falls in the range of 0 up to 4;

c falls in the range of 0 up to 4; and

d falls in the range of 0 up to 3.

As employed herein, the phrase "CAR or CAR-like isoform" refers to a member of the steroid/thyroid superfamily of receptors which is optionally constitutively active, and has at least 75 % overall amino acid identity (up to 86 % sequence similarity) with the receptor set forth in SEQ ID NO:1 (CAR-α), at least 88 % amino acid identity (up to 91 % sequence similarity) in the DNA binding domain thereof, with respect to the DNA binding domain of the receptor set forth in SEQ ID NO:1, and at least 74 % amino acid identity (up to 87 % sequence similarity) in the ligand binding domain thereof, with respect to the ligand binding domain of the receptor set forth in SEQ ID NO:1.

As employed herein, the phrase "modulating the activity of a CAR or CAR-like isoform" refers to the ability of a modulator (e.g., a ligand or precursor thereof) for an isoform of CAR or a CAR-like species to induce expression of gene(s) maintained under hormone expression control, or to repress expression of gene(s) maintained under such control.

As employed herein, the phrase "processes mediated by an isoform of CAR or a CAR-like species" refers to biological, physiological, endocrinological, and other bodily processes which are mediated by receptor or receptor combinations which are responsive to natural or synthetic androstans. Modulation of such processes can be accomplished *in vitro* or *in vivo*. *In vivo* modulation can

be carried out in a wide range of subjects, such as, for example, humans, rodents, sheep, pigs, cows, and the like.

As employed herein, "lower alkyl" refers to straight or branched chain alkyl groups having in the range of about 1 up to 4 carbon atoms; "alkyl" refers to straight or branched chain alkyl groups having in the range of about 1 up to 12 carbon atoms; "substituted alkyl" refers to alkyl groups further bearing one or more substituents such as hydroxy, alkoxy (of a lower alkyl group), mercapto (of a lower alkyl group), aryl, carboxyl, heterocyclic, halogen, trifluoromethyl, cyano, nitro, amino, carboxyl, carbamate, sulfonyl, sulfonamide, and the like.

As employed herein, "acyl" refers to alkyl-carbonyl groups.

Presently preferred compounds employed in the practice of the present invention include those wherein R^1 of structure I is hydrogen and R^2 is α-OR (wherein R is as defined above, with R = hydrogen or acyl being especially preferred); compounds wherein R^3 of structure I is methyl; compounds wherein R^4 of structure I is methyl; compounds according to structure I wherein $R^5 = R^6 = O$; compounds wherein R^5 and R^6 of structure I are both hydrogen; compounds wherein R^6 of structure I is absent, and there is a double bond between C^{16} and C^{17}, and the like.

In accordance with another embodiment of the present invention, there are provided methods for the identification of compounds which modulate the activity of a CAR or CAR-like isoform (as defined herein), said method comprising:

contacting host cell(s) containing receptor-encoded DNA and a suitable hormone response element linked to reporter-encoded DNA with test compound, and

determining the effect of test compound on the level of expression of said reporter.

Optionally, the receptor-encoded DNA employed in the practice of the present invention will also encode one or more exogenous transactivation domains, such as, for example, the τ_1 or τ_2 transactivation domains described in United States Patent No. 5,217,867, which is incorporated by reference herein in its entirety.

Those of skill in the art can readily determine suitable response elements for use in the practice of the present invention, such as, for example, the response elements described in United States Patent No. 5,091,518 and PCT published application no. WO 92/16546, both of which are hereby incorporated by reference herein.

Identification methods according to the present invention involve the use of a functional bioassay system, wherein the CAR or CAR-like isoform (as defined herein) and a reporter plasmid are cultured in suitable host cells in the presence of test compound. Evidence of transcription (e.g., expression) of reporter gene is then monitored to determine the presence of an activated receptor-ligand complex. Accordingly, the functional bioassay system utilizes two plasmids: an "expression" plasmid and a "reporter" plasmid. The expression plasmid can be any plasmid which contains and is capable of expressing DNA encoding the CAR or CAR-like isoform receptor protein, in a suitable host cell. The reporter plasmid can be any plasmid which contains an operative hormone response element functionally linked to an operative reporter gene.

Exemplary reporter genes include chloramphenicol transferase (CAT), luciferase (LUC), beta-galactosidase (β-gal), and the like. Exemplary promoters include the simian virus (SV) promoter or modified form thereof (e.g.,

ΔSV), the thymidine kinase (TK) promoter, the mammary tumor virus (MTV) promoter or modified form thereof (e.g., ΔMTV), and the like [see, for example, Mangelsdorf et al., in Nature 345:224-229 (1990), Mangelsdorf et al., in Cell 66:555-561 (1991), and Berger et al., in J. Steroid Biochem. Molec. Biol. 41:733-738 (1992)]. The plasmids pGMCAT, pGHCAT, and the like, are examples of reporter plasmids which contain an operative hormone responsive promoter/enhancer element functionally linked to an operative reporter gene, and can therefore be used in the above-described functional bioassay (see Example 1 for details on the preparation of these plasmids). In pGMCAT, the operative hormone responsive promoter/enhancer element is the MTV LTR; in pGHCAT it is the functional portion of the growth hormone promoter. In both pGMCAT and GHCAT the operative reporter gene is the bacterial gene for chloramphenicol acetyltransferase (CAT).

As used herein in the phrase "operative response element functionally linked to an operative reporter gene", the word "operative" means that the respective DNA sequences (represented by the terms "hormone response element" and "reporter gene") are operational, i.e., work for their intended purposes; the word "functionally" means that after the two segments are linked, upon appropriate activation by a ligand-receptor complex, the reporter gene will be expressed as the result of the fact that the "hormone response element" was "turned on" or otherwise activated.

In practicing the above-described functional bioassay, the expression plasmid and the reporter plasmid are co-transfected into suitable host cells. The transfected host cells are then cultured in the presence and absence of a test compound to determine if the test compound is able to produce activation of the promoter operatively linked to the hormone response element of the

reporter plasmid. Thereafter, the transfected and cultured host cells are monitored for induction (i.e., the presence) of the product of the reporter gene sequence.

Cells contemplated for use in the practice of the present invention include transformed cells, non-transformed cells, neoplastic cells, primary cultures of different cell types, and the like. Exemplary cells which can be employed in the practice of the present invention include liver cell lines (e.g., Hep-G2), primary hepatocytes, adipocyte or pre-adipocyte cell lines (e.g., 3T3-L1 cells, 3T3-442-A cells, OB17 cells, and the like), as well as CV-1 cells, HuTu80 cells, F9 cells, NTERA2 cells, NB4 cells, HL-60 cells, 293 cells, Hela cells, NIH-3T3 cells, and the like. Preferred host cells for use in the functional bioassay system are COS cells and CV-1 cells. COS-1 (referred to as COS) cells are monkey kidney cells that express SV40 T antigen (Tag); while CV-1 cells do not express SV40 Tag. The presence of Tag in the COS-1 derivative lines allows the introduced expression plasmid to replicate and provides a relative increase in the amount of receptor produced during the assay period. CV-1 cells are presently preferred because they are particularly convenient for gene transfer studies and provide a sensitive and well-described host cell system.

The above-described cells (or fractions thereof) are maintained under physiological conditions when contacted with physiologically active compound. "Physiological conditions" are readily understood by those of skill in the art to comprise an isotonic, aqueous nutrient medium at a temperature of about 37°C.

In accordance with yet another embodiment of the present invention, there is provided a method to increase the libido of a subject, said method comprising inhibiting the activity of CAR or CAR-like isoforms (as defined

above). In a particular aspect the above-described method to increase libido can be carried out by administering to a subject a libido-enhancing amount of a steroid-like compound having the structure I, as described herein.

Since individual subjects may present a wide variation in severity of symptoms and each drug has its unique therapeutic characteristics, the precise mode of administration and dosage employed for each subject is left to the discretion of the practitioner.

In accordance with still another embodiment of the present invention, there are provided physiologically active composition(s) comprising a compound having the structure I, as described herein, in a suitable vehicle rendering said compound amenable to oral delivery, transdermal delivery, intravenous delivery, intramuscular delivery, topical delivery, nasal delivery, and the like.

Pharmaceutical compositions of the present invention can be used in the form of a solid, a solution, an emulsion, a dispersion, a micelle, a liposome, and the like, wherein the resulting composition contains one or more of the compounds of the present invention, as an active ingredient, in admixture with an organic or inorganic carrier or excipient suitable for enteral or parenteral applications. The active ingredient may be compounded, for example, with the usual non-toxic, pharmaceutically acceptable carriers for tablets, pellets, capsules, suppositories, solutions, emulsions, suspensions, and any other form suitable for use. The carriers which can be used include glucose, lactose, gum acacia, gelatin, mannitol, starch paste, magnesium trisilicate, talc, corn starch, keratin, colloidal silica, potato starch, urea, medium chain length triglycerides, dextrans, and other carriers suitable for use in manufacturing preparations, in solid, semisolid, or liquid form. In addition auxiliary,

stabilizing, thickening and coloring agents and perfumes may be used. The active compound (i.e., compounds of structure I as described herein) is included in the pharmaceutical composition in an amount sufficient to produce the desired effect upon the process or condition of diseases.

Pharmaceutical compositions containing the active ingredient may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs. Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents selected from the group consisting of a sweetening agent such as sucrose, lactose, or saccharin, flavoring agents such as peppermint, oil of wintergreen or cherry, coloring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets containing the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients may also be manufactured by known methods. The excipients used may be, for example, (1) inert diluents such as calcium carbonate, lactose, calcium phosphate or sodium phosphate; (2) granulating and disintegrating agents such as corn starch, potato starch or alginic acid; (3) binding agents such as gum tragacanth, corn starch, gelatin or acacia, and (4) lubricating agents such as magnesium stearate, stearic acid or talc. The tablets may be uncoated or they may be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate may be employed. They may also be coated by the techniques described in the U.S. Pat. Nos. 4,256,108;

4,160,452; and 4,265,874, to form osmotic therapeutic tablets for controlled release.

In some cases, formulations for oral use may be in the form of hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin. They may also be in the form of soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example, peanut oil, liquid paraffin, or olive oil.

The pharmaceutical compositions may be in the form of a sterile injectable suspension. This suspension may be formulated according to known methods using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides, fatty acids (including oleic acid), naturally occurring vegetable oils like sesame oil, coconut oil, peanut oil, cottonseed oil, etc., or synthetic fatty vehicles like ethyl oleate or the like. Buffers, preservatives, antioxidants, and the like can be incorporated as required.

Compounds contemplated for use in the practice of the present invention may also be administered in the form of suppositories for rectal administration of the drug. These compositions may be prepared by mixing the drug with a suitable non-irritating excipient, such as cocoa butter, synthetic glyceride esters of polyethylene glycols, which are solid at ordinary temperatures, but liquify and/or dissolve in the rectal cavity to release the drug.

Since individual subjects may present a wide variation in severity of symptoms and each drug has its unique therapeutic characteristics, it is up to the practitioner to determine a subject's response to treatment and vary the dosages accordingly.

Typical daily doses, in general, lie within the range of from about 0.5 μg to about 10 mg per kg body weight, and, preferably within the range of from 50 μg to 1 mg per kg body weight and can be administered up to four times daily. The daily IV dose lies within the range of from about 1 μg to about 10 mg per kg body weight, and, preferably, within the range of from 10 μg to 500 μg per kg body weight.

In an alternate aspect of this embodiment of the present invention, compositions useful for ameliorating the libido-reducing effects of a 5α-reductase inhibitor are provided. Such compositions comprise a libido-enhancing amount of a steroid-like compound having the structure **I**, as described herein, and a 5α-reductase inhibitor.

Those of skill in the art can readily identify 5α-reductase inhibitors suitable for use in the practice of the present invention. An example of a 5α-reductase inhibitors contemplated for use in the practice of the present invention is finasteride (PROSCAR).

Since individual subjects may present a wide variation in severity of symptoms and each drug has its unique therapeutic characteristics, the precise mode of administration and dosage employed for each subject is left to the discretion of the practitioner.

In accordance with yet another embodiment of the present invention, there is provided a method for ameliorating the libido-reducing effects of a 5α-reductase

inhibitor, said method comprising co-administering, to a subject being treated with 5α-reductase inhibitor(s), a libido-enhancing amount of a steroid-like compound having the structure I, as described herein.

In accordance with a still further embodiment of the present invention, there is provided a method of screening cells or cell extracts to determine the presence of receptors involved in the modulation of libido, said method comprising

contacting cells or cell extracts with a compound having the structure I, as described herein, and thereafter

identifying those cells or cell extracts which bind said compound.

The invention will now be described in greater detail by reference to the following non-limiting examples.

Example 1
Preparation of reporter constructs

Various reporter constructs are used in the examples which follow. They are prepared as follows:

TK-LUC: The MTV-LTR promoter sequence is removed from the MTV-LUC plasmid described by Hollenberg and Evans in Cell 55:899-906 (1988) by *Hin*dIII and *Xho*I digest, and cloned with the *Hin*dIII-*Xho*I fragment of the Herpes simplex virus thymidine kinase gene promoter (-105 to +51 with respect to the transcription start site, m, isolated from plasmid pBLCAT2, described by Luckow & Schutz in Nucleic Acids Res. 15:5490 (1987)) to generate parental construct TK-LUC.

pTK-βRARE$_{1,2,3}$-LUC: One, two or three copies of double-stranded beta-retinoic acid response element (βRARE) oligonucleotides, comprising a direct repeat of two half sites separated by a spacer of five nucleotides, wherein each half site comprises the sequence

N_x-RGBNNM-,

wherein

R is selected from A or G;

B is selected from G, C, or T;

each N is independently selected from A, T, C, or G;

M is selected from A or C; and

x falls in the range of 0 up to 5;

with the proviso that at least 4 nucleotides of said -RGBNNM- sequence are identical with the nucleotides at corresponding positions of the sequence -AGGTCA-, is cloned upstream of the TK promoter of TK-LUC at the *Hin*dIII site.

Alternatively, response elements having a similar structure to that set forth above, except having a spacer of only four nucleotides, can be used. Thus, response elements comprising a direct repeat of two half sites separated by a spacer of four nucleotides, wherein each half site comprises the sequence

N_x-RGBNNM-,

as described above, can be used in place of the βRARE described above.

CMX-βGAL: The coding sequence for the *E. coli* β-galactosidase gene is isolated from plasmid pCH110 [see Hall et al., J. Mol. Appl. Genet. 2:101-109 (1983)] by *Hin*dIII and *Bam*HI digest, and cloned into pCMX eucaryotic expression vector [see Umesono et al., supra].

Example 2
Screening for CAR or CAR-like isoforms

A. With PCR-generated probe

A probe spanning the DNA-binding domain of the CAR-encoding DNA described by Baes et al. (Mol. and Cell. Biol. 14:1544-1552 (1994); i.e., nucleic acid residues 303 to 545 of SEQ ID NO:1) is prepared by PCR. The probe is labeled by the random-primer labeling method or by PCR using ^{32}P nucleotides. The labeled probe is then used to probe a lambda-gt11 mammalian liver cDNA library (e.g., mouse liver cDNA library or other readily available library, such as are commercially available from Clontech or Stratagene) to identify related receptors. The hybridization mixture contains 35% formamide, 1X Denhart's, 5X SSPE (1X SSPE = 0.15 M NaCl, 10mM Na_2HPO_4 1mM EDTA), 0.1% SDS, 10% dextran sulfate, 100 μg/ml denatured salmon sperm DNA and 10^6 cpm of [^{32}P]-labelled probe. Duplicate nitrocellulose filters are hybridized for 16h at 42°C, washed once at 25°C for 15 min with 2X SSC (1X SSC=0.15 M NaCl, 0.015 M sodium citrate), 0.1% SDS and then washed twice at 55°C for 30 min. in 2X SSC, 0.1% SDS. The filters are autoradiographed for 3 days at -70°C using an intensifying screen.

After several rounds of screening, several positive clones are obtained. Sequence analysis of at least one of the positive clones indicates that this clone encodes a novel member of the steroid/thyroid superfamily of receptors, having approximately 75 % overall amino acid identity with the receptor set forth in SEQ ID NO:1, approximately 88 % amino acid identity in the DNA binding domain thereof, with respect to the DNA binding domain of the receptor set forth in SEQ ID NO:1, and approximately 74 % amino acid identity in the ligand binding domain thereof,

with respect to the ligand binding domain of the receptor set forth in SEQ ID NO:1.

If the initial clone isolated is a partial clone, then an insert of the above-identified positive clone (labeled with ^{32}P) is also used as a probe to rescreen the same library or additional library(ies). Hybridization conditions for such rescreening comprise a hybridization mixture containing 50% formamide, 1X Denhart's, 5X SSPE, 0.1% SDS, 100 μg/ml denatured salmon sperm DNA and 10^6 cpm of [^{32}P]-labelled probe. Duplicate nitrocellulose filters are hybridized for 16h at 42°C, washed once at 60°C for 15 min with 0.1X SSC (1X SSC = 0.15 M NaCl, 0.015 M sodium citrate), 0.1% SDS and then washed twice at 60°C for 30 min. in 0.1X SSC, 0.1% SDS. The filters are autoradiographed for 3 days at -70°C using an intensifying screen.

After several rounds of screening, several positive clones are obtained.

B. <u>With synthetic oligonucleotides</u>

A lambda-gt11 mammalian liver cDNA library is screened in duplicate with a ^{32}P-labeled synthetic oligonucleotide:

TGYGARGGNT GYAARGGNTC TTT (SEQ ID NO:3),

under low-stringency conditions (i.e., 1M NaCl/0.05mM Tris-HCl, pH 8.0/5mM EDTA/150 units of heparin per ml/0.05%, sodium pyrophosphate/100 μg of yeast RNA per ml/0.1% (wt/vol) $NaDodSO_4$ at 46°C) and washed at high stringency, as described by Burglin et al., in Nature <u>341</u>:239-243 (1989). In the above oligonucleotide, Y is selected from C or T, R is selected from A or G, and N is any one of A, G, C or T. Thus, the oligonucleotide employed is a mixture of all possible DNA sequences encoding the amino acid sequence:

CEGCKGFF (SEQ ID NO:4),

wherein each letter above is the conventional single letter abbreviation for amino acid residues, i.e., C is cysteine, E is glutamic acid, G is glycine, K is lysine and F is phenylalanine.

Example 3

Screening assay for modulators of CAR or CAR-like isoforms

CV-1 cells are co-transfected with a vector encoding the CAR isoform isolated as described in Example 2 (incorporated into a CMV-driven expression vector), and pTK-βRARE-LUC at a ratio of about 100 ng of receptor-encoding DNA per 10^5 cells. The usual amounts of DNA per 10^5 cells are 100 ng of CDM8-CAR, 300 ng of pTK-βRARE-LUC, and 500 ng of CMX-βGAL. Typically, transfections are performed in triplicate. The plates are then incubated for 2-3 hours at 37°C.

The cells are washed with fresh medium. Fresh medium containing one concentration of a serial dilution of agonist is added to each well. A typical agonist dilution series extends from 10^{-5}M through 10^{-11}M. A solvent control is performed for each agonist. The cells are incubated at 37°C for 1-2 days.

The cells are rinsed twice with buffered saline solution. Subsequently, cells are lysed, *in situ*, by adding 200 µl of lysis buffer. After 30 minutes incubation at room temperature, 40 µl aliquots of cell lysate are transferred to 96-well plates for luciferase reporter gene assays and β-galactosidase transfection controls [see Heyman et al., Cell 68:397-406 (1992)].

The data are expressed as relative light units (RLUs) per O.D. unit of β-galactosidase per minute. The triplicates are averaged for each concentration and plotted

as normalized RLUs against the dose of agonist or as fold induction vs the dose of agonist. The results of testing with a variety of different compounds are presented in the following table:

Compound	Quantity	Relative light units
None	---	6.2
Androstenol	25 μM	0.3
Zaragozic acid	37 μM	7.2
Squelestatin	20 μM	6.5
Lovastatin	1 μM	6.2
Compactin	1 μM	5.8
Aminobenzotriazole	10 μM	9.3
Indomethacin	100 μM	7.1
Nordihydroquiaretic acid	50 μM	4.1
Squalene	10 μM	6.8
Retinoic acid	10 μM	5.9
Epiandrostenone + 5α-pregnenalone	@ 50 μM	3.4
Phenobarbitol	50 μM	7.5
Leukotriene B4	500 ng/ml	6.8
Prostaglandin E2	5 μg/ml	6.9
Octanoic acid	400 μM	8.5
t-β-carotene	5 μM	7.1
Farnesol	50 μM	10.2
Pregnenalone	50 μM	8.0
Cholesterol	50 μM	7.4
Arachidonic acid	30 μM	4.6
5-Hydroxyeicostetraenoic acid + 15-Hydroxyeicostetraenoic acid(R)	@ 500 ng/ml	7.0
8-Hydroxyeicostetraenoic acid(R,S)	500 ng/ml	8.5
25-OH-cholesterol	10 μM	5.9

Compound	Quantity	Relative light units
Vitamin K1/K2	@ 2.5 μM	7.6
reverse triiodothyronine	5 μM	8.8
Anhydro-retinol	50 μM	6.8
14-OH-retroretinol	1.4 μM	8.0
Taurocholic acid + Taurodeoxycholic acid	@ 200 μM	5.8
Dehydroepiandrostenone	50 μM	5.9
Vitamin E	50 μM	6.8

This example demonstrates the androstans (such as androstenol) are effective at reducing the constitutive activity of the CAR isoform employed herein.

The selectivity of a modulator for a particular receptor can be measured by comparing the activation/repression of that receptor with the activation/repression of some other related receptor with the same modulator.

Example 4

Dose response of CAR or CAR-like isoforms to modulators therefor

Effector plasmid, reporter plasmid, and β-galactosidase control plasmid are co-transfected into CV-1 cells at a ratio of about 1:3:5, using a liposome-mediated method, employing N-{1-(2,3-dioleoyloxy)propyl-N,N,N-trimethyl ammonium methyl sulfate} (i.e., DOTAP (Boehringer Manheim) according to manufacturer's instructions in Dulbecco's modified Eagle's medium (DMEM) with 10% delipidated hormone-depleted fetal calf serum. After about 2-3 hours, the cells are washed twice with fresh DMEM and test compound is added to the media to the final molar concentration indicated in Figure 1. After

24-48 hours of incubation, the media is removed and the cells are lysed. Aliquots are assayed for luciferase and β-galactosidase activity. Luciferase activity is normalized to optical density units of β-galactosidase per minute of incubation.

The data are expressed in Figure 1 as the normalized response to solvent or test compound, relative to induction of the same construct incubated in solvent alone.

Review of Figure 1 reveals that the androstans (such as androstenol, androstenol-3-acetate, 5α-androstan-3α-ol, and the like) are effective at suppressing the constitutive activity of CAR or CAR-like isoforms, with androstenol and 5α-androstan-3α-ol being the presently preferred androstans for use in the practice of the present invention.

While the invention has been described in detail with reference to certain preferred embodiments thereof, it will be understood that modifications and variations are within the spirit and scope of that which is described and claimed.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Evans, Ronald M.
Forman, Barry M.

(ii) TITLE OF INVENTION: MODULATORS FOR NEW MEMBERS OF THE STEROID/THYROID SUPERFAMILY OF RECEPTORS

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Pretty, Schroeder, Brueggemann & Clark
(B) STREET: 444 South Flower Street, Suite 2000
(C) CITY: Los Angeles
(D) STATE: CA
(E) COUNTRY: USA
(F) ZIP: 90071

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/442,464
(B) FILING DATE: 16-MAY-1995
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Reiter, Stephen E.
(B) REGISTRATION NUMBER: 31,192
(C) REFERENCE/DOCKET NUMBER: P41 9881

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 619-546-4737
(B) TELEFAX: 619-546-9392

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1450 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 273..1319

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GTGAGCTTGC TCCTTAAGTT ACAGGAACTC TCCTTATAAT AGACACTTCA TTTTCCTAGT 60

CCATCCCTCA TGAAAAATGA CTGACCACTG CTGGGCAGCA GGAGGGATGA TAATCCTAAC 120

TCCAATCACT GGCAACTCCT GAGATCAGAG GAAAACCAGC AACAGCGTGG GAGTTTGGGG 180

AGAGGCATTC CATACCAGAT TCTGTGGCCT GCAGGTGACA TGCTGCCTAA GAGAAGCAGG 240

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AGTCTGTGAC AGCCACCCCA ACACGTGACG TC ATG GCC AGT AGG GAA GAT GAG          293
                                    Met Ala Ser Arg Glu Asp Glu
                                     1               5

CTG AGG AAC TGT GTG GTA TGT GGG GAC CAA GCC ACA GGC TAC CAC TTT          341
Leu Arg Asn Cys Val Val Cys Gly Asp Gln Ala Thr Gly Tyr His Phe
        10                  15                  20

AAT GCG CTG ACT TGT GAG GGC TGC AAG GGT TTC TTC AGG AGA ACA GTC          389
Asn Ala Leu Thr Cys Glu Gly Cys Lys Gly Phe Phe Arg Arg Thr Val
    25                  30                  35

AGC AAA AGC ATT GGT CCC ACC TGC CCC TTT GCT GGA AGC TGT GAA GTC          437
Ser Lys Ser Ile Gly Pro Thr Cys Pro Phe Ala Gly Ser Cys Glu Val
40                  45                  50                  55

AGC AAG ACT CAG AGG CGC CAC TGC CCA GCC TGC AGG TTG CAG AAG TGC          485
Ser Lys Thr Gln Arg Arg His Cys Pro Ala Cys Arg Leu Gln Lys Cys
                60                  65                  70

TTA GAT GCT GGC ATG AGG AAA GAC ATG ATA CTG TCG GCA GAA GCC CTG          533
Leu Asp Ala Gly Met Arg Lys Asp Met Ile Leu Ser Ala Glu Ala Leu
            75                  80                  85

GCA TTG CGG CGA GCA AAG CAG GCC CAG CGG CGG GCA CAG CAA ACA CCT          581
Ala Leu Arg Arg Ala Lys Gln Ala Gln Arg Arg Ala Gln Gln Thr Pro
        90                  95                  100

GTG CAA CTG AGT AAG GAG CAA GAA GAG CTG ATC CGG ACA CTC CTG GGG          629
Val Gln Leu Ser Lys Glu Gln Glu Glu Leu Ile Arg Thr Leu Leu Gly
    105                 110                 115

GCC CAC ACC CGC CAC ATG GGC ACC ATG TTT GAA CAG TTT GTG CAG TTT          677
Ala His Thr Arg His Met Gly Thr Met Phe Glu Gln Phe Val Gln Phe
120                 125                 130                 135

AGG CCT CCA GCT CAT CTG TTC ATC CAT CAC CAG CCC TTG CCC ACC CTG          725
Arg Pro Pro Ala His Leu Phe Ile His His Gln Pro Leu Pro Thr Leu
                140                 145                 150

GCC CCT GTG CTG CCT CTG GTC ACA CAC TTC GCA GAC ATC AAC ACT TTC          773
Ala Pro Val Leu Pro Leu Val Thr His Phe Ala Asp Ile Asn Thr Phe
            155                 160                 165

ATG GTA CTG CAA GTC ATC AAG TTT ACT AAG GAC CTG CCC GTC TTC CGT          821
Met Val Leu Gln Val Ile Lys Phe Thr Lys Asp Leu Pro Val Phe Arg
        170                 175                 180

TCC CTG CCC ATT GAA GAC CAG ATC TCC CTT CTC AAG GGA GCA GCT GTG          869
Ser Leu Pro Ile Glu Asp Gln Ile Ser Leu Leu Lys Gly Ala Ala Val
    185                 190                 195

GAA ATC TGT CAC ATC GTA CTC AAT ACC ACT TTC TGT CTC CAA ACA CAA          917
Glu Ile Cys His Ile Val Leu Asn Thr Thr Phe Cys Leu Gln Thr Gln
200                 205                 210                 215

AAC TTC CTC TGC GGG CCT CTT CGC TAC ACA ATT GAA GAT GGA GCC CGT          965
Asn Phe Leu Cys Gly Pro Leu Arg Tyr Thr Ile Glu Asp Gly Ala Arg
                220                 225                 230

GTG GGG TTC CAG GTA GAG TTT TTG GAG TTG CTC TTT CAC TTC CAT GGA         1013
Val Gly Phe Gln Val Glu Phe Leu Glu Leu Leu Phe His Phe His Gly
            235                 240                 245

ACA CTA CGA AAA CTG CAG CTC CAA GAG CCT GAG TAT GTG CTC TTG GCT         1061
Thr Leu Arg Lys Leu Gln Leu Gln Glu Pro Glu Tyr Val Leu Leu Ala
        250                 255                 260
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```
GCC ATG GCC CTC TTC TCT CCT GAC CGA CCT GGA GTT ACC CAG AGA GAT      1109
Ala Met Ala Leu Phe Ser Pro Asp Arg Pro Gly Val Thr Gln Arg Asp
    265                 270                 275

GAG ATT GAT CAG CTG CAA GAG GAG ATG GCA CTG ACT CTG CAA AGC TAC      1157
Glu Ile Asp Gln Leu Gln Glu Glu Met Ala Leu Thr Leu Gln Ser Tyr
280                 285                 290                 295

ATC AAG GGC CAG CAG CGA AGG CCC CGG GAT CGG TTT CTG TAT GCG AAG      1205
Ile Lys Gly Gln Gln Arg Arg Pro Arg Asp Arg Phe Leu Tyr Ala Lys
                300                 305                 310

TTG CTA GGC CTG CTG GCT GAG CTC CGG AGC ATT AAT GAG GCC TAC GGG      1253
Leu Leu Gly Leu Leu Ala Glu Leu Arg Ser Ile Asn Glu Ala Tyr Gly
            315                 320                 325

TAC CAA ATC CAG CAC ATC CAG GGC CTG TCT GCC ATG ATG CCG CTG CTC      1301
Tyr Gln Ile Gln His Ile Gln Gly Leu Ser Ala Met Met Pro Leu Leu
        330                 335                 340

CAG GAG ATC TGC AGC TGAGGCCATG CTCACTTCCT TCCCCAGCTC ACCTGGAACA      1356
Gln Glu Ile Cys Ser
    345

CCCTGGATAC ACTGGAGTGG GAAAATGCTG GGACCAAAGA TTGGGCCGGG TTCAAAGGGA    1416

GCCCAGTGGT TGCAATGAAA GACTAAAGCA AAAC                                1450
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 348 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Ala Ser Arg Glu Asp Glu Leu Arg Asn Cys Val Val Cys Gly Asp
  1               5                  10                  15

Gln Ala Thr Gly Tyr His Phe Asn Ala Leu Thr Cys Glu Gly Cys Lys
            20                  25                  30

Gly Phe Phe Arg Arg Thr Val Ser Lys Ser Ile Gly Pro Thr Cys Pro
        35                  40                  45

Phe Ala Gly Ser Cys Glu Val Ser Lys Thr Gln Arg Arg His Cys Pro
    50                  55                  60

Ala Cys Arg Leu Gln Lys Cys Leu Asp Ala Gly Met Arg Lys Asp Met
65                  70                  75                  80

Ile Leu Ser Ala Glu Ala Leu Ala Leu Arg Arg Ala Lys Gln Ala Gln
                85                  90                  95

Arg Arg Ala Gln Gln Thr Pro Val Gln Leu Ser Lys Glu Gln Glu Glu
            100                 105                 110

Leu Ile Arg Thr Leu Leu Gly Ala His Thr Arg His Met Gly Thr Met
        115                 120                 125

Phe Glu Gln Phe Val Gln Phe Arg Pro Pro Ala His Leu Phe Ile His
    130                 135                 140
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His Gln Pro Leu Pro Thr Leu Ala Pro Val Leu Pro Leu Val Thr His
145                 150                 155                 160

Phe Ala Asp Ile Asn Thr Phe Met Val Leu Gln Val Ile Lys Phe Thr
                165                 170                 175

Lys Asp Leu Pro Val Phe Arg Ser Leu Pro Ile Glu Asp Gln Ile Ser
            180                 185                 190

Leu Leu Lys Gly Ala Ala Val Glu Ile Cys His Ile Val Leu Asn Thr
        195                 200                 205

Thr Phe Cys Leu Gln Thr Gln Asn Phe Leu Cys Gly Pro Leu Arg Tyr
    210                 215                 220

Thr Ile Glu Asp Gly Ala Arg Val Gly Phe Gln Val Glu Phe Leu Glu
225                 230                 235                 240

Leu Leu Phe His Phe His Gly Thr Leu Arg Lys Leu Gln Leu Gln Glu
                245                 250                 255

Pro Glu Tyr Val Leu Leu Ala Ala Met Ala Leu Phe Ser Pro Asp Arg
            260                 265                 270

Pro Gly Val Thr Gln Arg Asp Glu Ile Asp Gln Leu Gln Glu Glu Met
        275                 280                 285

Ala Leu Thr Leu Gln Ser Tyr Ile Lys Gly Gln Gln Arg Arg Pro Arg
    290                 295                 300

Asp Arg Phe Leu Tyr Ala Lys Leu Leu Gly Leu Leu Ala Glu Leu Arg
305                 310                 315                 320

Ser Ile Asn Glu Ala Tyr Gly Tyr Gln Ile Gln His Ile Gln Gly Leu
                325                 330                 335

Ser Ala Met Met Pro Leu Leu Gln Glu Ile Cys Ser
            340                 345
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TGYGARGGNT GYAARGGNTC TTT 23

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

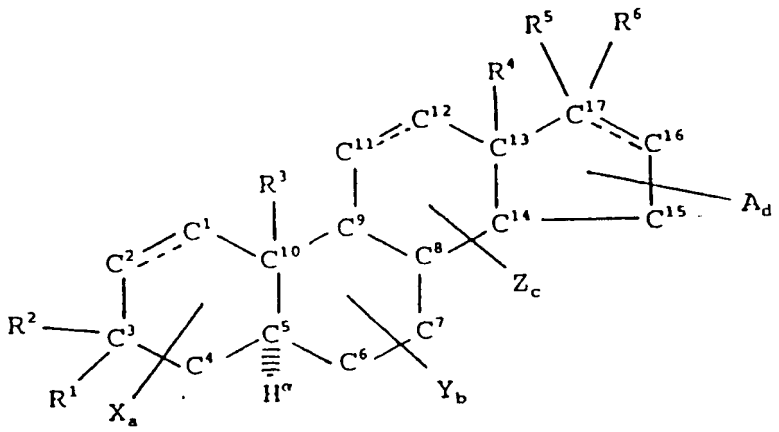
(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Cys Glu Gly Cys Lys Gly Phe Phe
1 5

That which is claimed is:

1. A method for modulating the activity of an isoform of CAR or a CAR-like species, said method comprising administering an effective amount of a steroid-like compound having the structure:



wherein:

$R^1 = R^2 = O$; or R^1 = hydrogen and

R^2 is α-OR, wherein R is selected from hydrogen, lower alkyl, acyl or trimethylsilyl;

R^3 and R^4 are each independently hydrogen or lower alkyl;

$R^5 = R^6 = O$; or R^5 and R^6 are both hydrogen; or R^6 is absent when there is a double bond between C^{16} and C^{17};

X, Y, Z and A are each independently selected from hydroxy, alkoxy (of a lower alkyl group), mercapto (of a lower alkyl group), halogen, trifluoromethyl, cyano, nitro, amino, carboxyl, carbamate, sulfonyl, sulfonamide;

a falls in the range of 0 up to 4;

b falls in the range of 0 up to 4;

c falls in the range of 0 up to 4; and

d falls in the range of 0 up to 3.

2. A method according to claim 1 wherein said isoform of CAR or CAR-like species is a member of the steroid/thyroid superfamily of receptors having at least 75 % overall amino acid homology with the receptor set forth in SEQ ID NO:1 (CAR-α), at least 88 % amino acid homology in the DNA binding domain thereof, with respect to the DNA binding domain of the receptor set forth in SEQ ID NO:1, and at least 74 % amino acid homology in the ligand binding domain thereof, with respect to the ligand binding domain of the receptor set forth in SEQ ID NO:1.

3. A method according to claim 1 wherein said isoform of CAR or a CAR-like species has at least 75 % overall amino acid identity with the receptor set forth in SEQ ID NO:1 (CAR-α), at least 88 % amino acid identity in the DNA binding domain thereof, with respect to the DNA binding domain of the receptor set forth in SEQ ID NO:1, and at least 74 % amino acid identity in the ligand binding domain thereof, with respect to the ligand binding domain of the receptor set forth in SEQ ID NO:1.

4. A method according to claim 1 wherein said member has at least 86 % overall amino acid similarity with the receptor set forth in SEQ ID NO:1 (CAR-α), at least 91 % amino acid similarity in the DNA binding domain thereof, with respect to the DNA binding domain of the receptor set forth in SEQ ID NO:1, and at least 87 % amino acid similarity in the ligand binding domain thereof, with respect to the ligand binding domain of the receptor set forth in SEQ ID NO:1.

5. A method according to claim 1 wherein R^1 is hydrogen and R^2 is α-OR, wherein R is as defined above.

6. A method according to claim 5 wherein R is hydrogen or acyl.

7. A method according to claim 1 wherein R^3 is methyl.

8. A method according to claim 1 wherein R^4 is methyl.

9. A method according to claim 1 wherein $R^5 = R^6 = O$.

10. A method according to claim 1 wherein R^5 and R^6 are both hydrogen.

11. A method according to claim 1 wherein R^6 is absent, and there is a double bond between C^{16} and C^{17}.

12. A method for the identification of compounds which modulate the activity of an isoform of CAR or a CAR-like species, said method comprising:

contacting host cell(s) containing receptor-encoded DNA and a suitable hormone response element linked to reporter-encoded DNA with test compound, and

determining the effect of test compound on the level of expression of said reporter.

13. A method according to claim 12 wherein said isoform of CAR or a CAR-like species is a member of the steroid/thyroid superfamily of receptors having at least 75 % overall amino acid homology with the receptor set forth in SEQ ID NO:1 (CAR-α), at least 88 % amino acid homology in the DNA binding domain thereof, with respect to the DNA binding domain of the receptor set forth in SEQ ID NO:1, and at least 74 % amino acid homology in the ligand binding domain thereof, with respect to the ligand binding domain of the receptor set forth in SEQ ID NO:1.

14. A method according to claim 12 wherein said response element is a direct repeat of two or more half sites separated by a spacer of four or five nucleotides, wherein each half site comprises the sequence

N_x-RGBNNM-,

wherein

R is selected from A or G;

B is selected from G, C, or T;

each N is independently selected from A, T, C, or G;

M is selected from A or C; and

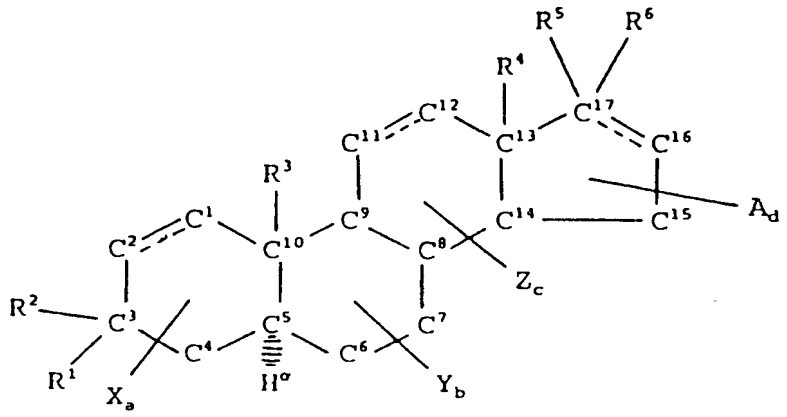
x falls in the range of 0 up to 5;

with the proviso that at least 4 nucleotides of said -RGBNNM- sequence are identical with the nucleotides at corresponding positions of the sequence -AGGTCA-.

15. A method to increase the libido of a subject, said method comprising modulating the activity of an isoform of CAR or a CAR-like species.

16. A method according to claim 15 wherein said isoform of CAR or a CAR-like species is a member of the steroid/thyroid superfamily of receptors having at least 75 % overall amino acid homology with the receptor set forth in SEQ ID NO:1 (CAR-α), at least 88 % amino acid homology in the DNA binding domain thereof, with respect to the DNA binding domain of the receptor set forth in SEQ ID NO:1, and at least 74 % amino acid homology in the ligand binding domain thereof, with respect to the ligand binding domain of the receptor set forth in SEQ ID NO:1.

17. A method to increase the libido of a subject, said method comprising administering to said subject a libido-enhancing amount of a steroid-like compound having the structure I as follows:



I

wherein:

$R^1 = R^2 = O$; or R^1 = hydrogen and

R^2 is α-OR, wherein R is selected from hydrogen, lower alkyl, acyl or trimethylsilyl;

R^3 and R^4 are each independently hydrogen or lower alkyl;

$R^5 = R^6 = O$; or R^5 and R^6 are both hydrogen; or R^6 is absent when there is a double bond between C^{16} and C^{17};

X, Y, Z and A are each independently selected from hydroxy, alkoxy (of a lower alkyl group), mercapto (of a lower alkyl group), halogen, trifluoromethyl, cyano, nitro, amino, carboxyl, carbamate, sulfonyl, sulfonamide;

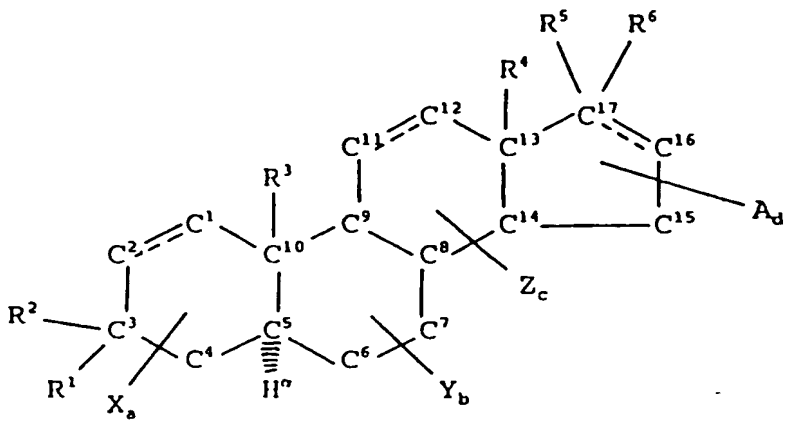
a falls in the range of 0 up to 4;

b falls in the range of 0 up to 4;

c falls in the range of 0 up to 4; and

d falls in the range of 0 up to 3.

18. A physiologically active composition comprising a compound having the structure I as follows:



I

wherein:

$R^1 = R^2 = O$; or R^1 = hydrogen and

R^2 is α-OR, wherein R is selected from hydrogen, lower alkyl, acyl or trimethylsilyl;

R^3 and R^4 are each independently hydrogen or lower alkyl;

$R^5 = R^6 = O$; or R^5 and R^6 are both hydrogen; or R^6 is absent when there is a double bond between C^{16} and C^{17};

X, Y, Z and A are each independently selected from hydroxy, alkoxy (of a lower alkyl group), mercapto (of a lower alkyl group), halogen, trifluoromethyl, cyano, nitro, amino, carboxyl, carbamate, sulfonyl, sulfonamide;

a falls in the range of 0 up to 4;

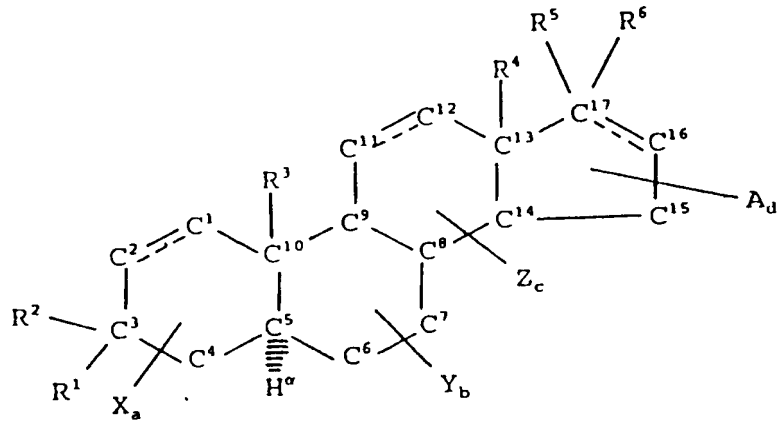
b falls in the range of 0 up to 4;

c falls in the range of 0 up to 4; and

d falls in the range of 0 up to 3

in a suitable vehicle rendering said compound amenable to oral, transdermal or nasal delivery.

19. A method for ameliorating the libido-reducing effects of a 5α-reductase inhibitor, said method comprising co-administering, to a subject being treated with 5α-reductase inhibitor(s), a libido-enhancing amount of a steroid-like compound having the structure I as follows:



I

wherein:

$R^1 = R^2 = O$; or R^1 = hydrogen and

R^2 is α-OR, wherein R is selected from hydrogen, lower alkyl, acyl or trimethylsilyl;

R^3 and R^4 are each independently hydrogen or lower alkyl;

$R^5 = R^6 = O$; or R^5 and R^6 are both hydrogen; or R^6 is absent when there is a double bond between C^{16} and C^{17};

X, Y, Z and A are each independently selected from hydroxy, alkoxy (of a lower alkyl group), mercapto (of a lower alkyl group), halogen, trifluoromethyl, cyano, nitro, amino, carboxyl, carbamate, sulfonyl, sulfonamide;

a falls in the range of 0 up to 4;

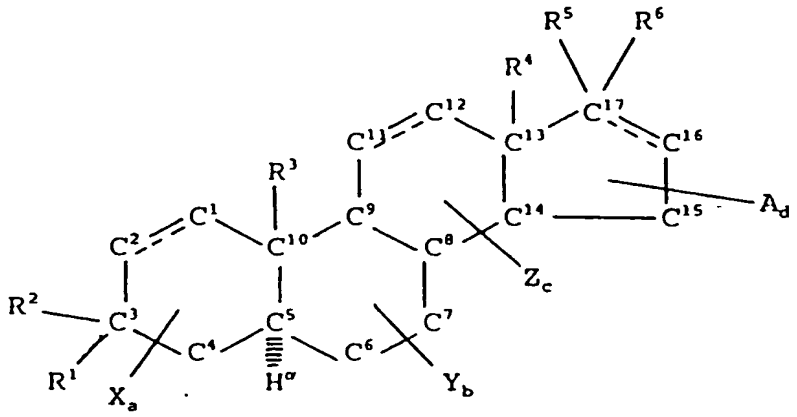
b falls in the range of 0 up to 4;

c falls in the range of 0 up to 4; and

d falls in the range of 0 up to 3.

20. A method according to claim 19 wherein said 5α-reductase inhibitor is finasteride (PROSCAR).

21. A composition comprising a 5α-reductase inhibitor and a libido-enhancing amount of a steroid-like compound having the structure I as follows:



I

wherein:

$R^1 = R^2 = O$; or R^1 = hydrogen and

R^2 is α-OR, wherein R is selected from hydrogen, lower alkyl, acyl or trimethylsilyl;

R^3 and R^4 are each independently hydrogen or lower alkyl;

$R^5 = R^6 = O$; or R^5 and R^6 are both hydrogen; or R^6 is absent when there is a double bond between C^{16} and C^{17};

X, Y, Z and A are each independently selected from hydroxy, alkoxy (of a lower alkyl group), mercapto (of a lower alkyl group), halogen, trifluoromethyl, cyano, nitro, amino, carboxyl, carbamate, sulfonyl, sulfonamide;

a falls in the range of 0 up to 4;

b falls in the range of 0 up to 4;

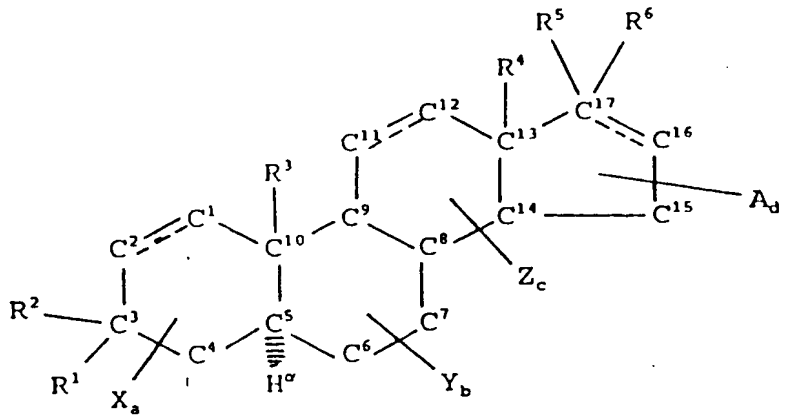
c falls in the range of 0 up to 4; and

d falls in the range of 0 up to 3.

22. A composition according to claim 21 wherein said 5α-reductase inhibitor is finasteride (PROSCAR).

23. Method of screening cells or cell extracts to determine the presence of receptors involved in the modulation of libido, said method comprising

contacting cells or cell extracts with a compound having the structure I as follows:



I

wherein:

$R^1 = R^2 = O$; or R^1 = hydrogen and

R^2 is α-OR, wherein R is selected from hydrogen, lower alkyl, acyl or trimethylsilyl;

R^3 and R^4 are each independently hydrogen or lower alkyl;

$R^5 = R^6 = O$; or R^5 and R^6 are both hydrogen; or R^6 is absent when there is a double bond between C^{16} and C^{17};

X, Y, Z and A are each independently selected from hydroxy, alkoxy (of a lower alkyl group), mercapto (of a lower alkyl group), halogen, trifluoromethyl, cyano, nitro, amino, carboxyl, carbamate, sulfonyl, sulfonamide;

a falls in the range of 0 up to 4;

b falls in the range of 0 up to 4;
c falls in the range of 0 up to 4; and
d falls in the range of 0 up to 3,

and thereafter

identifying those cells or cell extracts which bind said compound.

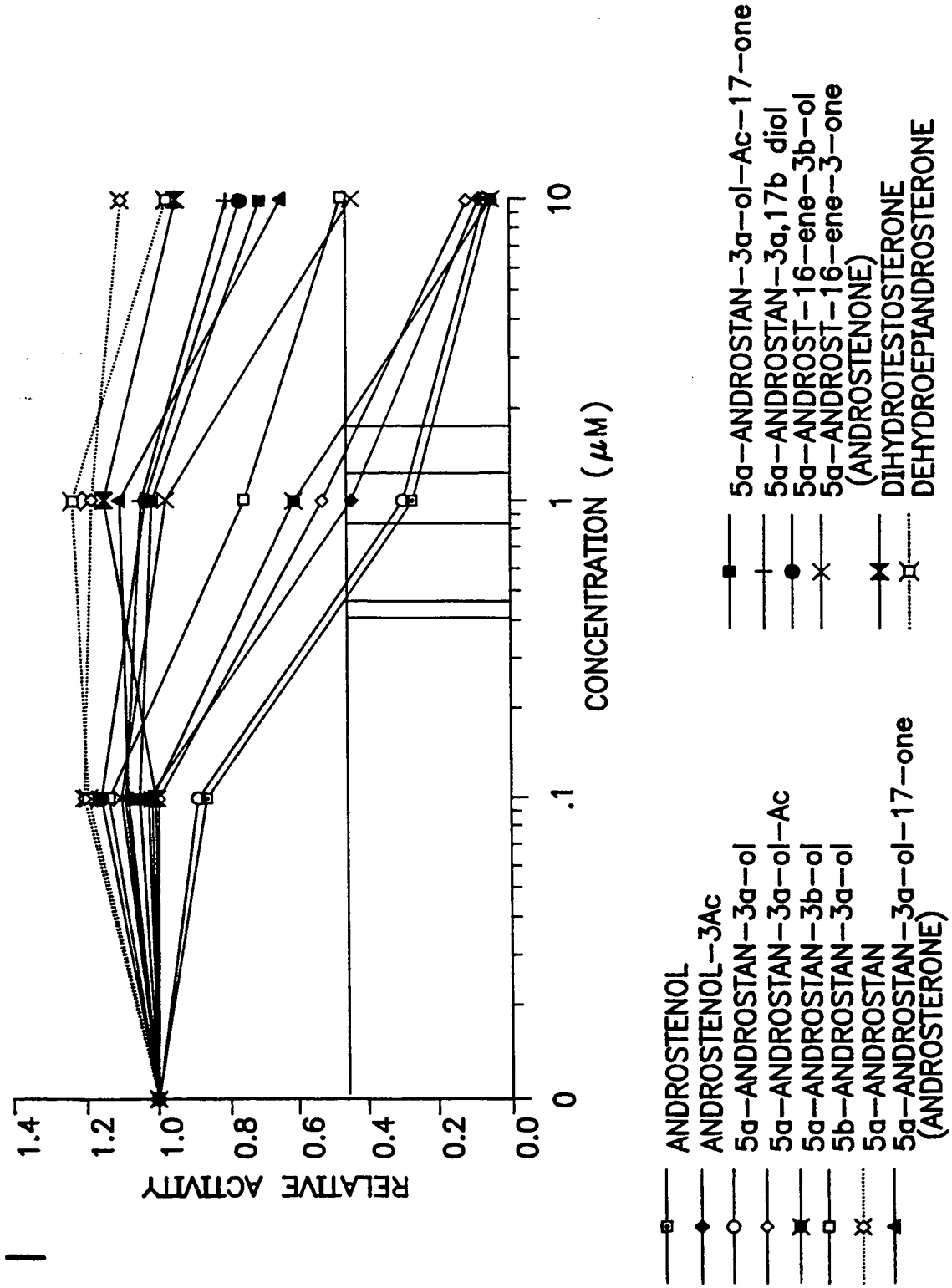

RELATIVE ACTIVITY
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
.1
1
10
CONCENTRATION (μM)
ANDROSTENOL
ANDROSTENOL-3Ac
5α-ANDROSTAN-3α-ol
5α-ANDROSTAN-3α-ol-Ac
5α-ANDROSTAN-3b-ol
5b-ANDROSTAN-3α-ol
5α-ANDROSTAN
5α-ANDROSTAN-3α-ol-17-one (ANDROSTERONE)
5α-ANDROSTAN-3α-ol-Ac-17-one
5α-ANDROSTAN-3α,17b diol
5α-ANDROST-16-ene-3b-ol
5α-ANDROST-16-ene-3-one (ANDROSTENONE)
DIHYDROTESTOSTERONE
DEHYDROEPIANDROSTERONE

FIG. 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/03865

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A16K 31/56, 31/365; G01N 33/53, 33/566
US CL :514/177, 178, 179, 180, 182; 435/7.1, 7.21, 7.8
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/177, 178, 179, 180, 182; 435/7.1, 7.21, 7.8

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, HCAPLUS, REGISTRY.
search terms: retino?, stero?, androst? Moore.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BAES et al. A new orphan member of the nuclear hormone receptor superfamily that interacts with a subset of retinoic acid response elements. Molecular and cellular biology. March 1994, Vol. 14, No. 3, pages 1544-1552, see entire document.	1-11
A, P	MANGELSDORF et al. The nuclear receptor superfamily: the second decade. Cell. 15 December 1995, Vol. 83, pages 835-839, see entire document.	1-11
A, P	MANGELSDORF et al. The RXR heterodimers and orphan receptors. Cell. 15 December 1995, Vol. 83, pages 841-850, see entire document.	1-11

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
02 JULY 1996

Date of mailing of the international search report
27 AUG 1996

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PCT/US96/03865

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HEYMAN et al. 9-cis retinoic acid is a high affinity ligand for the retinoic x receptor. Cell. 24 January 1992, Vol. 68, pages 397-406, see entire document.	1-11
A, P	FORMAN et al. Identification of a nuclear receptor that is activated by farnesol metabolites. Cell. 02 June 1995, Vol. 81, pages 687-693, see entire document.	1-11
X	BENNUA-SKALMOWSKI et al. A facile conversion of primary or secondary alcohols with n-perfluorobutane-sulfonyl fluoride/1,8-diazabicyclo[5.4.0]undec-7-ene into their corresponding fluroides. Tetrahedron letters. 10 April 1995, Vol. 36, pages 2611-2614, see entire document.	18

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Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-11, 18

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-11, 18 drawn to a method for modulating the activity of an isoform of CAR and a physiological active composition.

Group II: Claims 12-14 drawn to a method for the identification of compounds which modulate the activity of an isoform of CAR.

Group III: Claims 15-16 drawn to a method to increase the libido of a subject, said method comprising modulating the activity of an isoform of CAR.

Group IV: Claim 17 drawn to a method to increase the libido of a subject, said method comprising administering to said subject a libido-enhancing amount of a steroid-like compound.

Group V: Claims 19-20 a method for ameliorating the libido-reducing effects of a 5-alpha-reductase.

Group VI: Claims 21-22 drawn to a composition of 5-alpha-reductase inhibitor and a libido-enhancing amount of a steroid like compound.

Group VII: Claim 23 drawn to a method of screening cells.

The inventions listed as Groups I-VII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons. Group I is drawn to a method for modulating the activity of an isoform of CAR and a physiological active composition comprising a compound having the structure I. The special technical feature of Group I is the method of modulating the activity of an isoform of CAR or a CAR-like species by administering an effective amount of a steroid like compound. Group VI is drawn to another composition of 5-alpha-reductase inhibitor and a libido-enhancing amount of a steroid like compound. Group VI special technical feature is the 5-alpha-reductase inhibitor and is different from group I. Groups II-V, and VII are drawn to different methods of using the receptor or the steroid like compound structure I which do not share the same or corresponding special technical feature as group I. Note that PCT Rule 13 does not provide for multiple products or methods within a single application. Since the special technical feature of each group invention is not present in any other group invention, unity of invention is lacking.